Predicting the Bioavailability of PAHs from MGP Soils and Sediments Using Mild Supercritical CO$_2$ Extraction

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Observation: Based on 14 MGP soils and soots, and 43 MGP sediments, acute toxicity to soil and benthic organisms from PAHs has virtuallly **_NO_** relationship to total PAH concentrations.

Soil	Total PAH (mg/kg soil)	% Worm mortality
OG10	42100	0 (all lived)
CG3	4100	100 (all died)
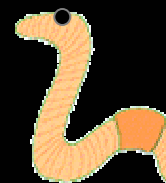
Goals:

To develop extraction conditions for "bioavailable" molecules only.

To use this method to predict exposure and uptake.

To gain regulatory acceptance for risk assessment.

Talk outline

MGP vs. Petroleum and definition of "total" PAHs.

Why supercritical carbon dioxide (SFE)?

Comparison of SFE to bioremediation.

Comparison of SFE to water desorption.

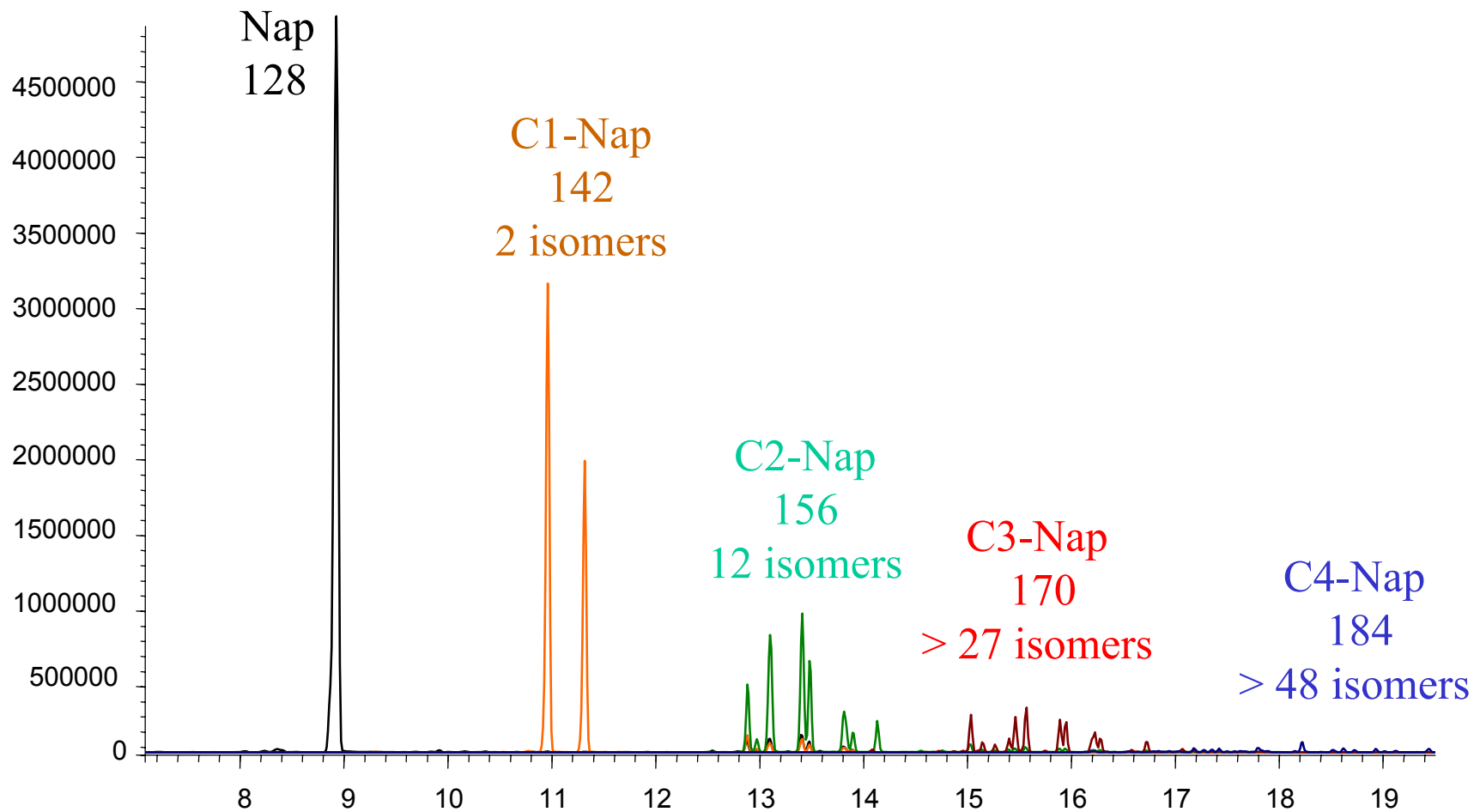**Comparison of SFE to organisms uptake and toxicity.**

EPA is moving from "total" PAHs based on 16 parent PAHs to "34" PAHs parent and alkyl PAHs.

This will affect petroleum PAH concentrations more than MGP PAH concentrations.
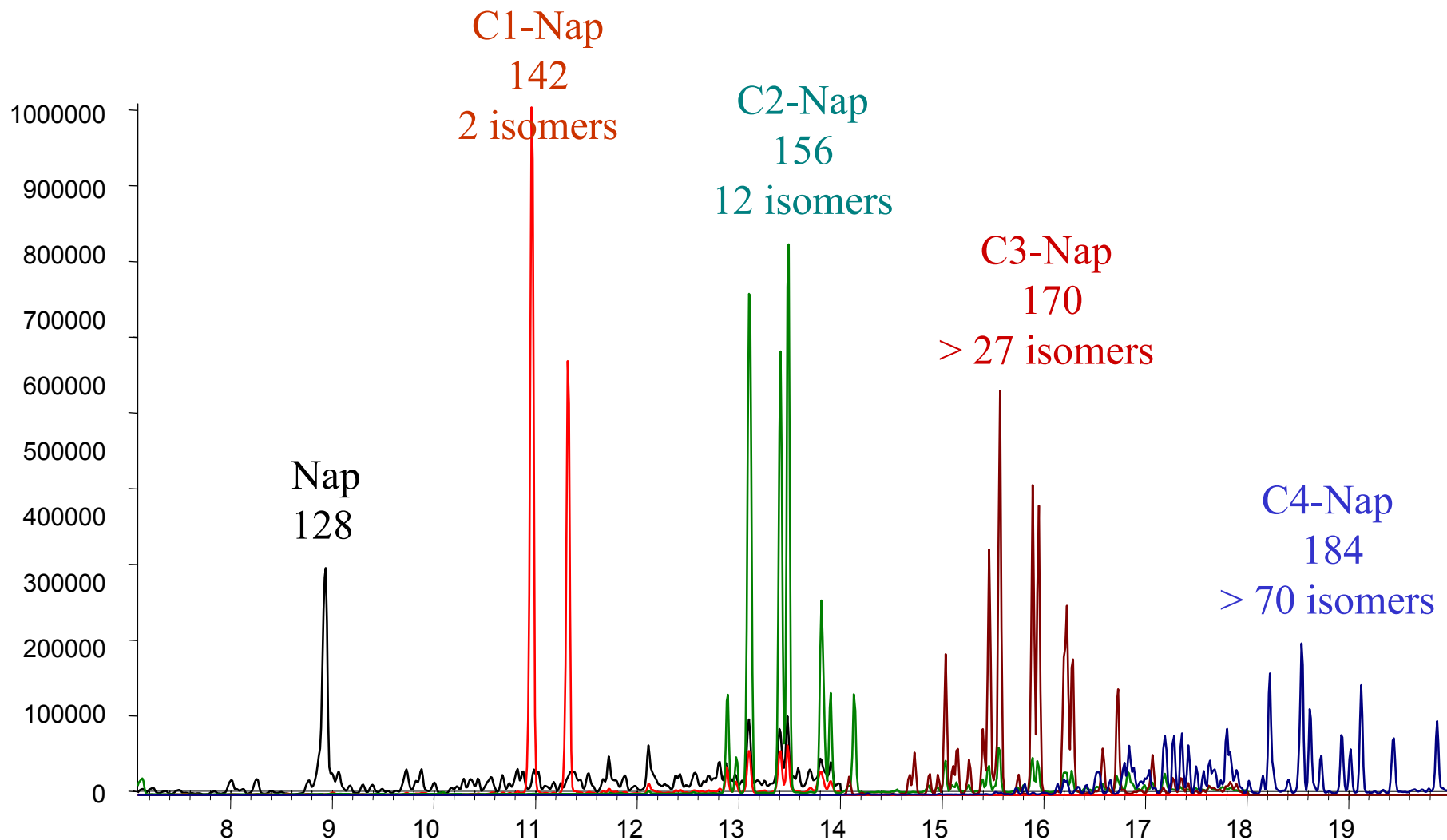
MGP vs. Petroleum Hydrocarbons

	parent/alkyl PAHs	alkanes
MGP sites (50)	40% parent PAHs	much lower than PAHs
NIST crude oil	1% parent PAHs	much higher than PAHs

Naphthalenes in MGP Sediment

Nap
128

C1-Nap
142
2 isomers

C2-Nap
156
12 isomers

C3-Nap
170
> 27 isomers

C4-Nap
184
> 48 isomers

Naphthalenes in Petroleum Crude Oil

Nap
128

C1-Nap
142
2 isomers

C2-Nap
156
12 isomers

C3-Nap
170
> 27 isomers

C4-Nap
184
> 70 isomers

Effect of alkyl PAHs on "total" PAH concentrations

Total "priority pollutant" PAHs measures only the 16 parent PAHs.

Total "34" PAHs measures 16 parent and 18 groups of alkyl PAHs.

So what? If the true PAH concentration was 1000 mg/kg, "total" PAH concentration based on "16" parent PAHs would be:

> *>> approximately 400 mg/kg for MGP.*

> *>> approximately 10 mg/kg for petroleum (based on NIST crude oil).*

*The present talk will focus on PAHs from MGP sites.*

>>soils, soots, and sediments

>>results should apply to other hydrophobic pollutants (PCBs, dioxins, many pesticides, etc.)

Approach: Compare mild SFE to:

1. Bioremediation of PAHs in a field site (training set).

2. Compare SFE to water desorption (12 soils and soots).

3. Live worms/dead worms (removal of toxicity by mild SFE).

4. Earthworm PAH uptake and toxicity.

5. Water flea toxicity (*Hyalella*)

6. Water worm uptake (*Lumbriculus*)

7. Mice uptake

All Samples Used Are from "Real" Sites Typically Abandoned 50 Years Ago
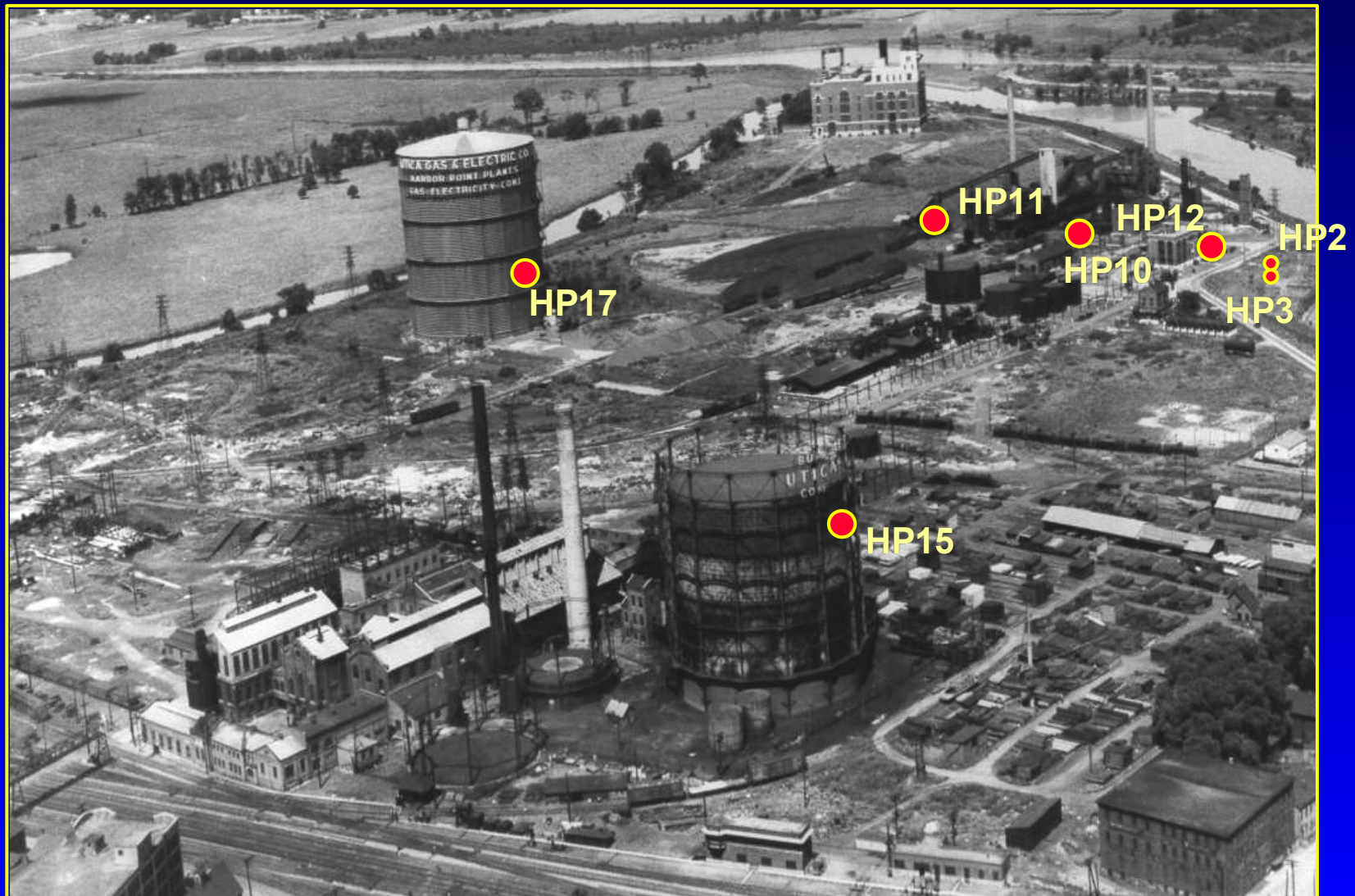
Nyack, NY MGP Site

What is SFE (Supercritical Fluid Extraction)?

- High pressure CO_2 is pumped through a sample, and extracted organics are collected in a suitable solvent for GC analysis.

- Typical SFE conditions:
  - 100 to 400 bar (density of ca. 0.4 to 0.9 g/mL)
  - 40 to 150 ^{o}C
  - 1 mL/min with a 1 to 10-gram sample

Why use supercritical carbon dioxide?

1. *CO_2 is a lipophilic solvent much like biological lipids in polarity*

2. *PAH solubilities in CO_2 are proportional to those in water, but ca. 10^4 higher.*

Characteristics of Common Aromatics: CO_2 Solubility Mimics Water Solubility

Compound	# Rings	Boiling pt.	Solubility	
			water	CO_2 (50 °C, 400 bar)
			(g/kg X 10^4)	(g/kg)
benzene	1	80 °C	18000	miscible
naphthalene	2	218 °C	320	120
phenanthrene	3	340 °C	13	11
pyrene	4	394 °C	1.4	1.2
chrysene	5	448 °C	0.02	0.02
benzo[ghi]perylene	6	500 °C	0.003	0.002

Test Case #1: Bioremediation of PAHs

on an MGP Soil

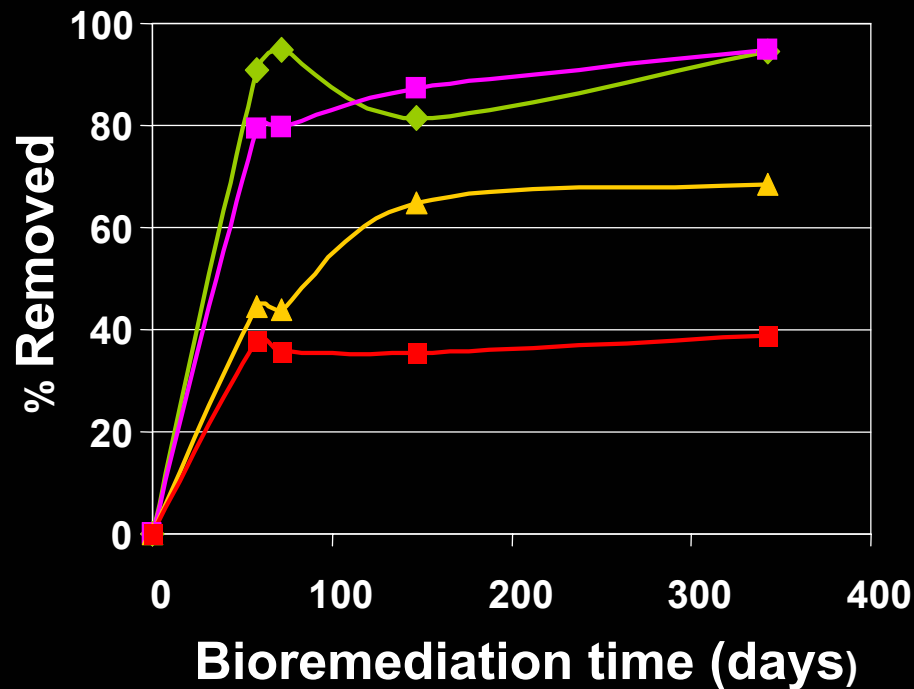*Site: Soccer-sized field, ca. 50 cm deep*

Treatment: Bioremediation with tillage and nutrients for one year.

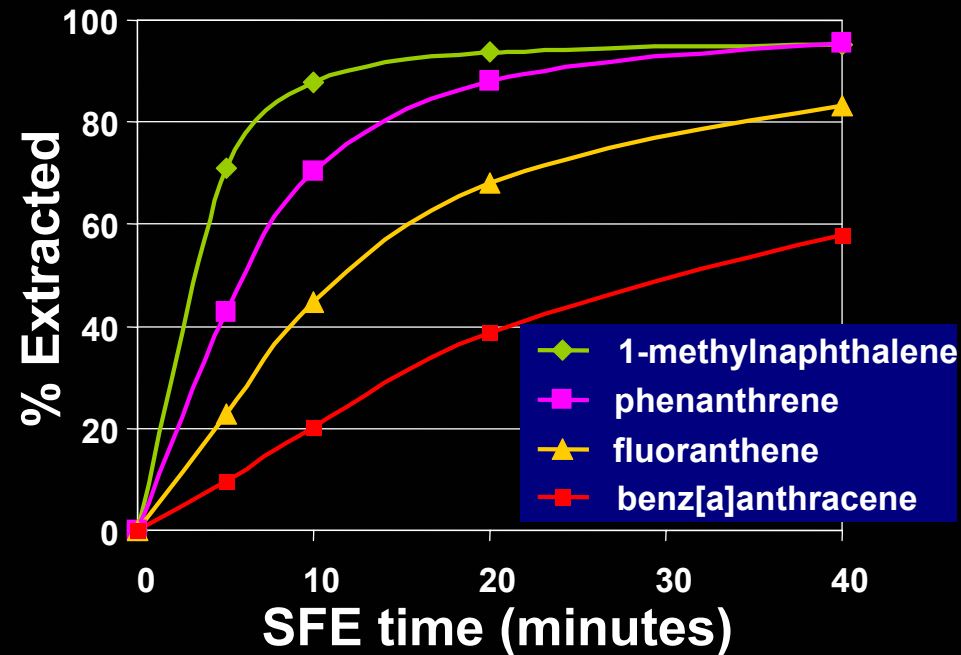*PAHs: 2 to 6 ring (MW from 128 to 278),*

7000 mg/kg

Approach: Develop SFE conditions that mimic bioremediation progress. (150 to 400 bar, 40 to 150 ^{o}C)

SFE rates (single condition) mimic bioremediation (1 min SFE=10 days bioremediation)
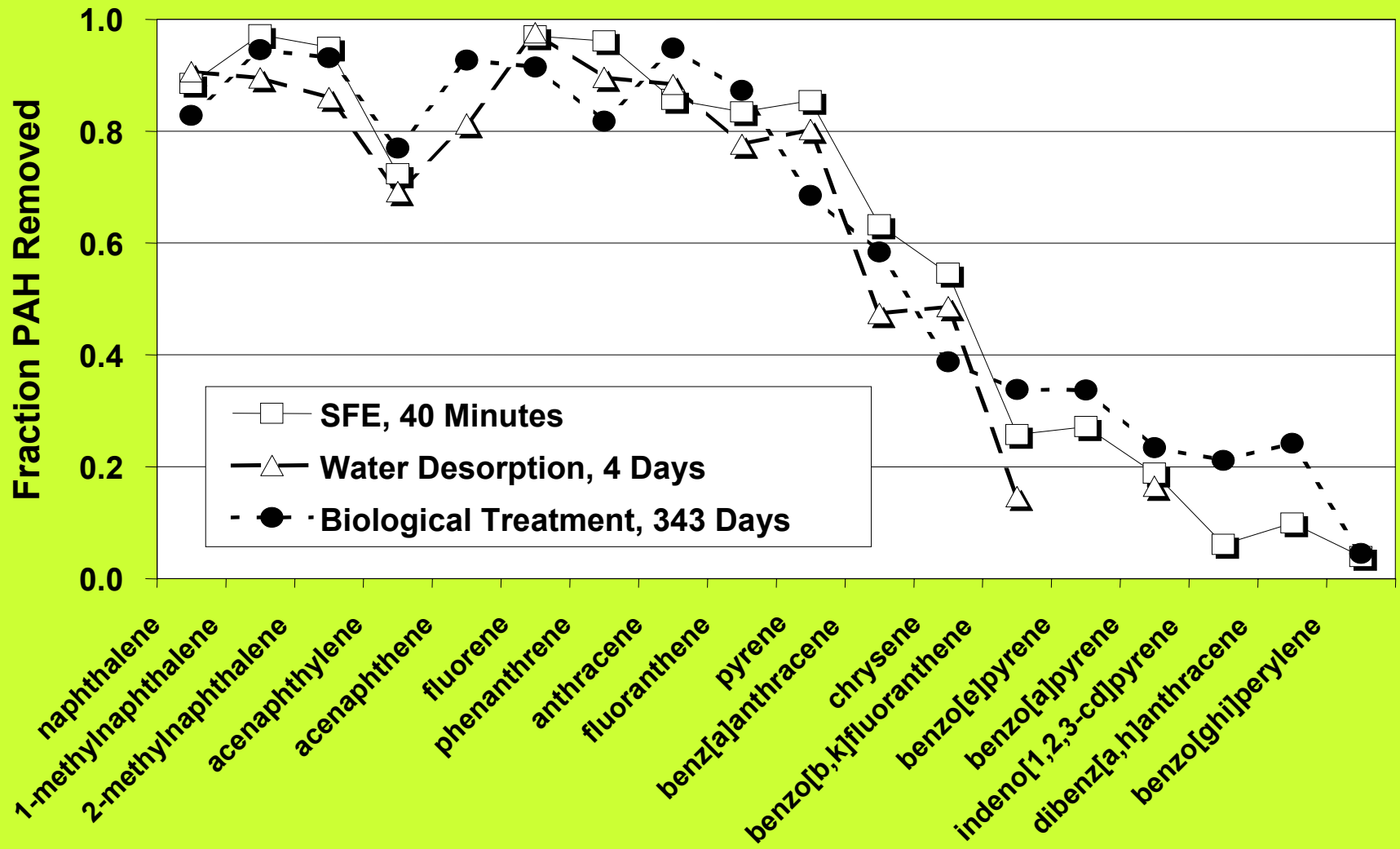

Bioremediation (1 year)

SFE (40 min.)

Legend:
- 1-methylnaphthalene
- phenanthrene
- fluoranthene
- benz[a]anthracene

Left chart: Y-axis "% Removed" (0–100), X-axis "Bioremediation time (days)" (0–400)

Right chart: Y-axis "% Extracted" (0–100), X-axis "SFE time (minutes)" (0–40)

Samples provided by GTI and MidAmerican Energy

40 min of SFE predicts the result of one year of field bioremediation (PAHs ranging from MW 128 to 276)

Test Case 2: Compare SFE to water desorption.

SFE conditions were developed on samples from the field bioremediation studies described earlier.

These "magic" conditions (200 bar, 50 °C) were tested with soils and soots from 14 sites.

> *2- 6 ring PAHs*

> *150 to 40000 mg/kg total concentrations*

> *2 to 87 wt.% carbon*

Result: 120 minutes SFE agreed with 120 days of water desorption on the amount of each PAH that was "available." (16 PAHs X 12 samples)
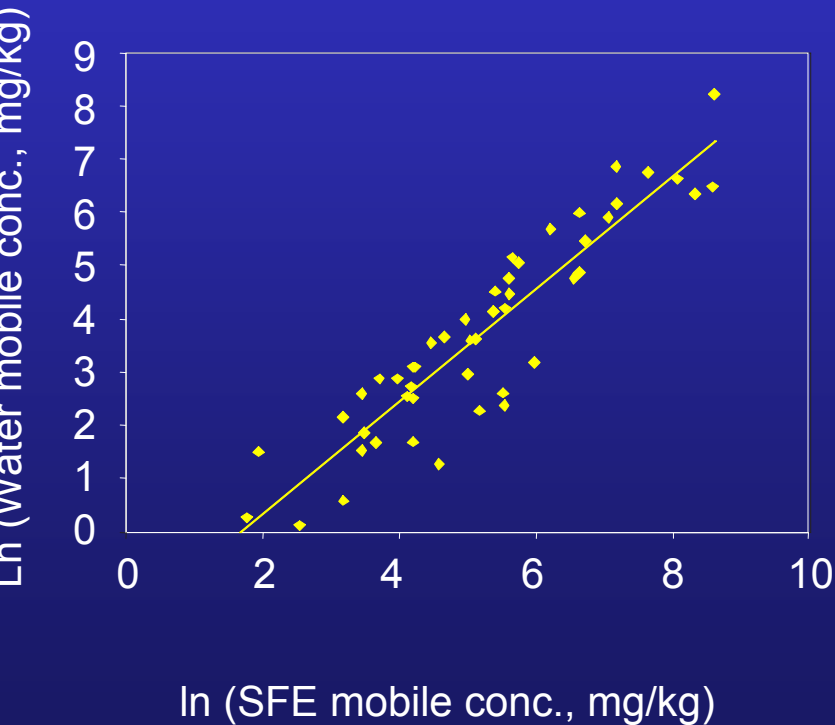
>> $r^2 = 0.84$

Comparison of Mobile PAH *Concentrations*

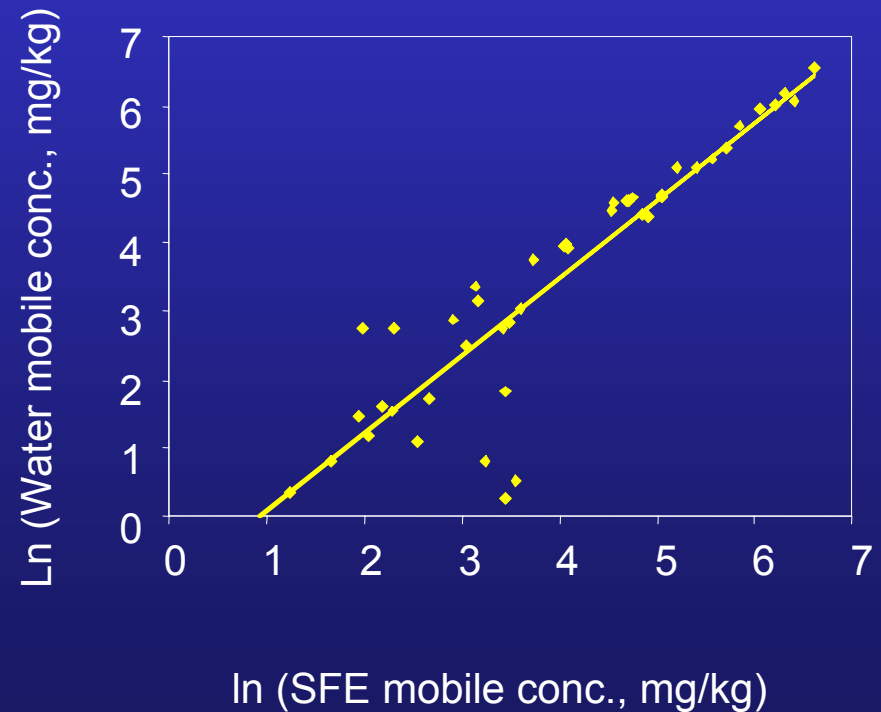## SFE and water desorption agree fairly well for both oil gas and coal gas sampl

Coal Gas Samples

ln (water mobile conc.) = 1.05 ln (SFE mobile conc.) - 1.76
$r^2 = 0.84$

Ln (Water mobile conc., mg/kg)

ln (SFE mobile conc., mg/kg)

Oil Gas Samples

ln (water mobile conc.) = 1.14 ln (SFE mobile conc.) - 1.04
$r^2 = 0.82$

Ln (Water mobile conc., mg/kg)

ln (SFE mobile conc., mg/kg)

Test Case #3: SFE vs. Worm PAH Uptake Bioassay

- **14 - 28 day test**
- **20 gm soil**
- **5 worms**
- **No toxicity**

Earthworm Uptake Soil Characteristics

Worms survived in the samples below !

Soil	Total Elemental Carbon	Total PAHs
	(%)	(mg/kg soil)
CG15	24.1	1,020
OG14	2.9	168
CG11	29.3	15,600
CG12	7.9	3,790
OG17	47.3	17,210
OG5	6.9	1,870
OG10	86.6	42,100

Sediment 15
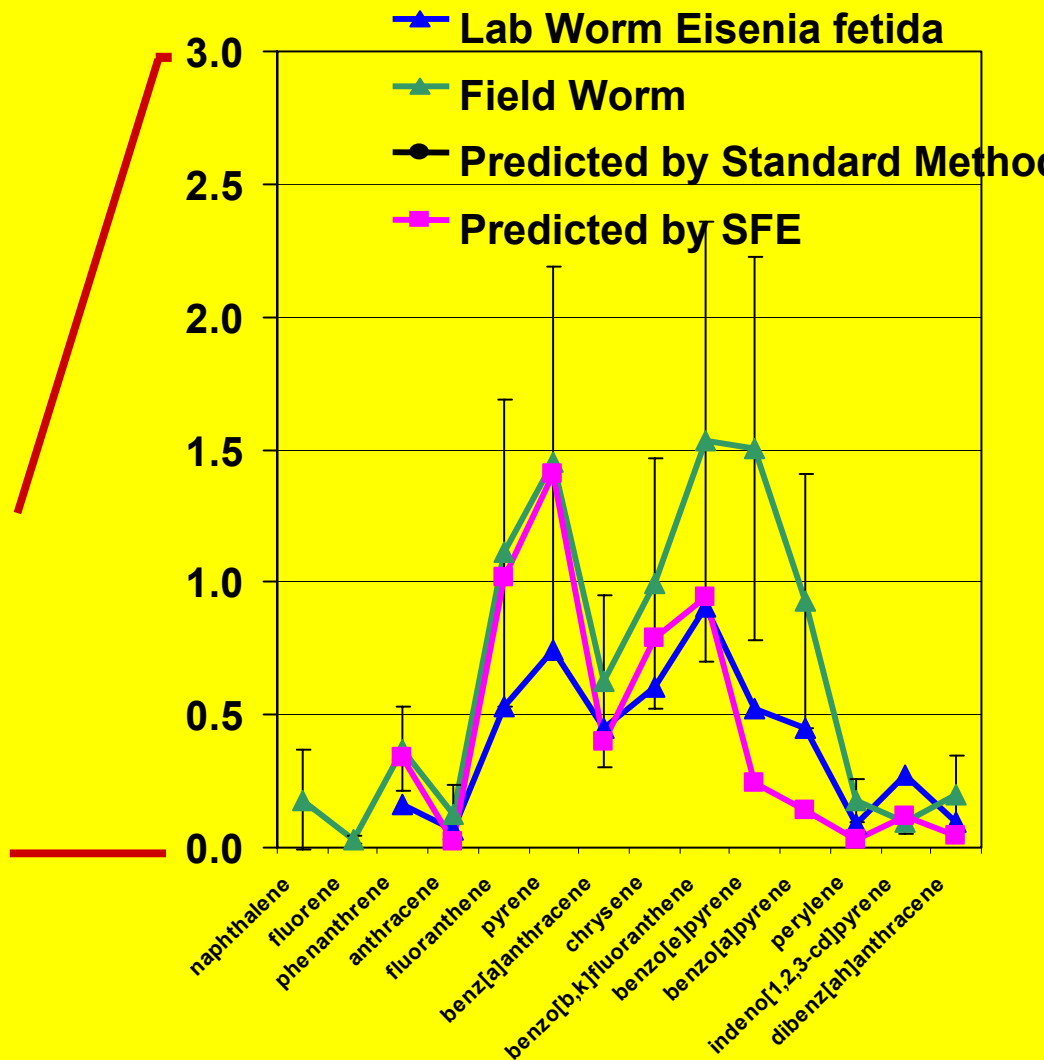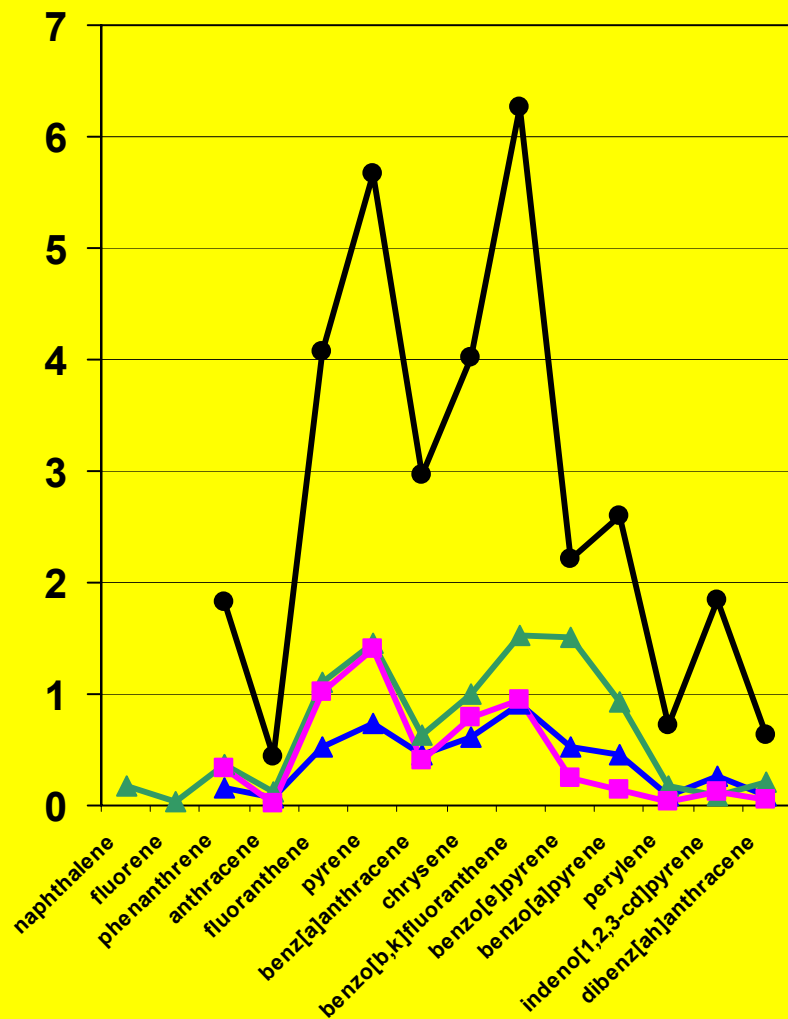
12 soils X 16 PAHs

The Regulatory Model Overpredicts Worm Uptake for all PAHs from all Samples up to 1000X
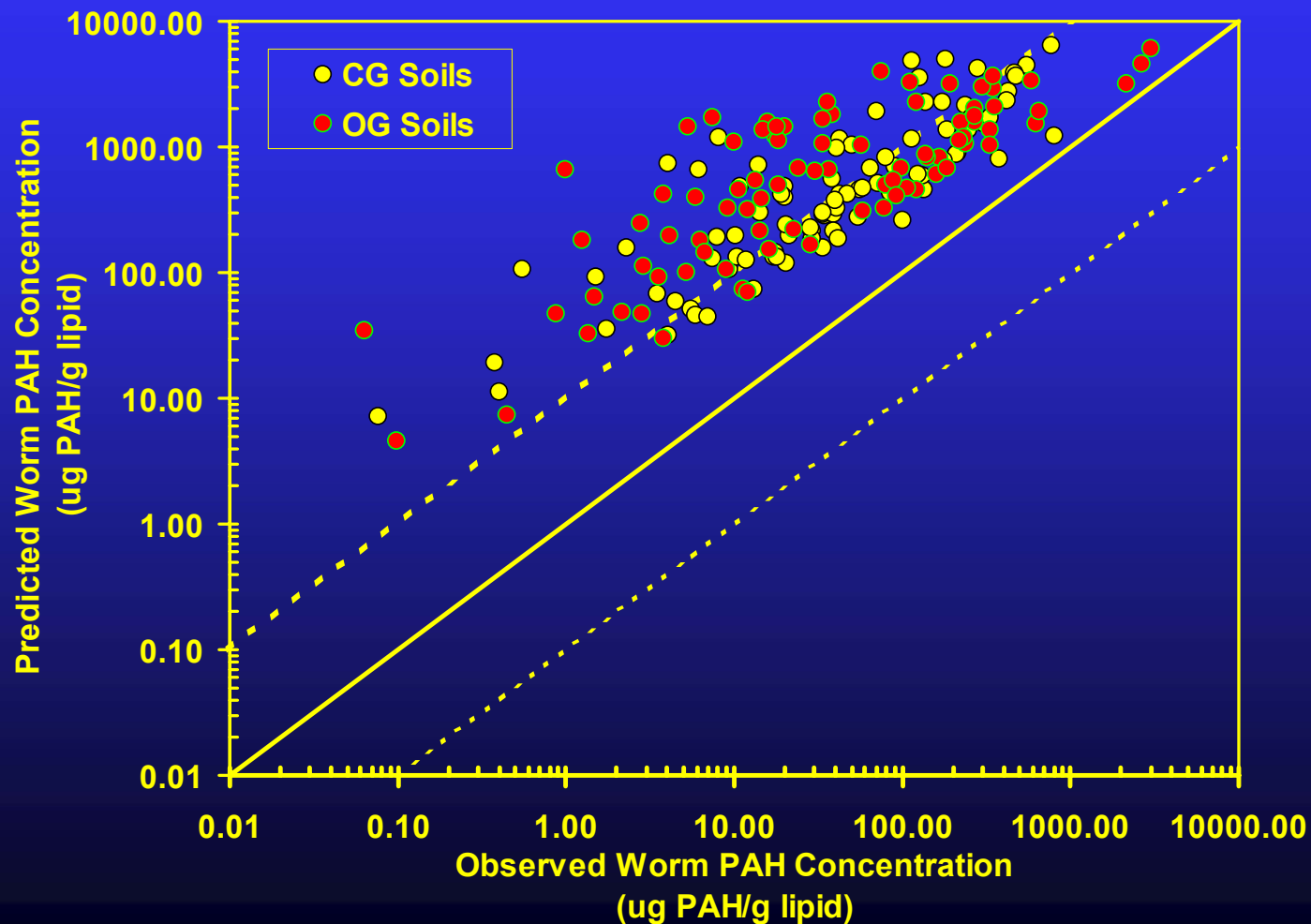
Legend:
- CG Soils
- OG Soils

Y-axis: Predicted Worm PAH Concentration (ug PAH/g lipid)

X-axis: Observed Worm PAH Concentration (ug PAH/g lipid)
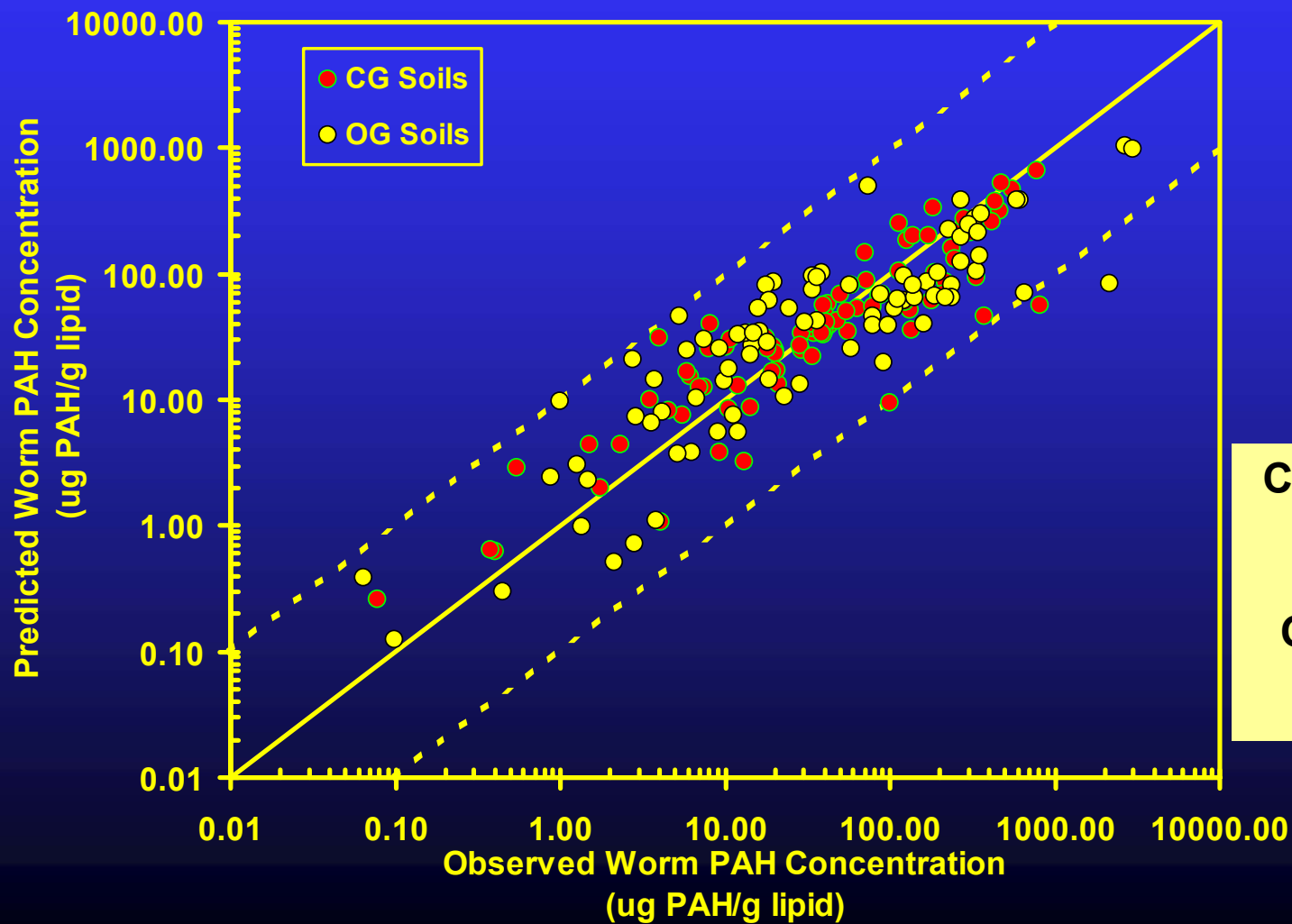
Earthworm Mortality Depends on Available PAHs (measured by SFE), Not on Total PAH Concentrations

Soil	Total PAH (ug/g soil)	Available Fraction (SFE)	Available Total PAH (ug/g C)	% Mortality
CG15	1020	0.25	1040	0
OG14	168	0.46	2720	0
CG11	15600	0.06	3280	0
CG12	3790	0.16	7880	0
OG17	17200	0.27	9720	0
OG5	1870	0.41	11100	0
OG10	42100	0.33	16300	0
CG3	4100	0.83	45700	100
OG18	17300	0.74	50100	100

Test case 4: SFE "available" benzo[a]pyrene correlates with uptake in mice.

$y = 11.476x + 0.0789$

$R^2 = 0.9838$

In Vitro, % b[a]p
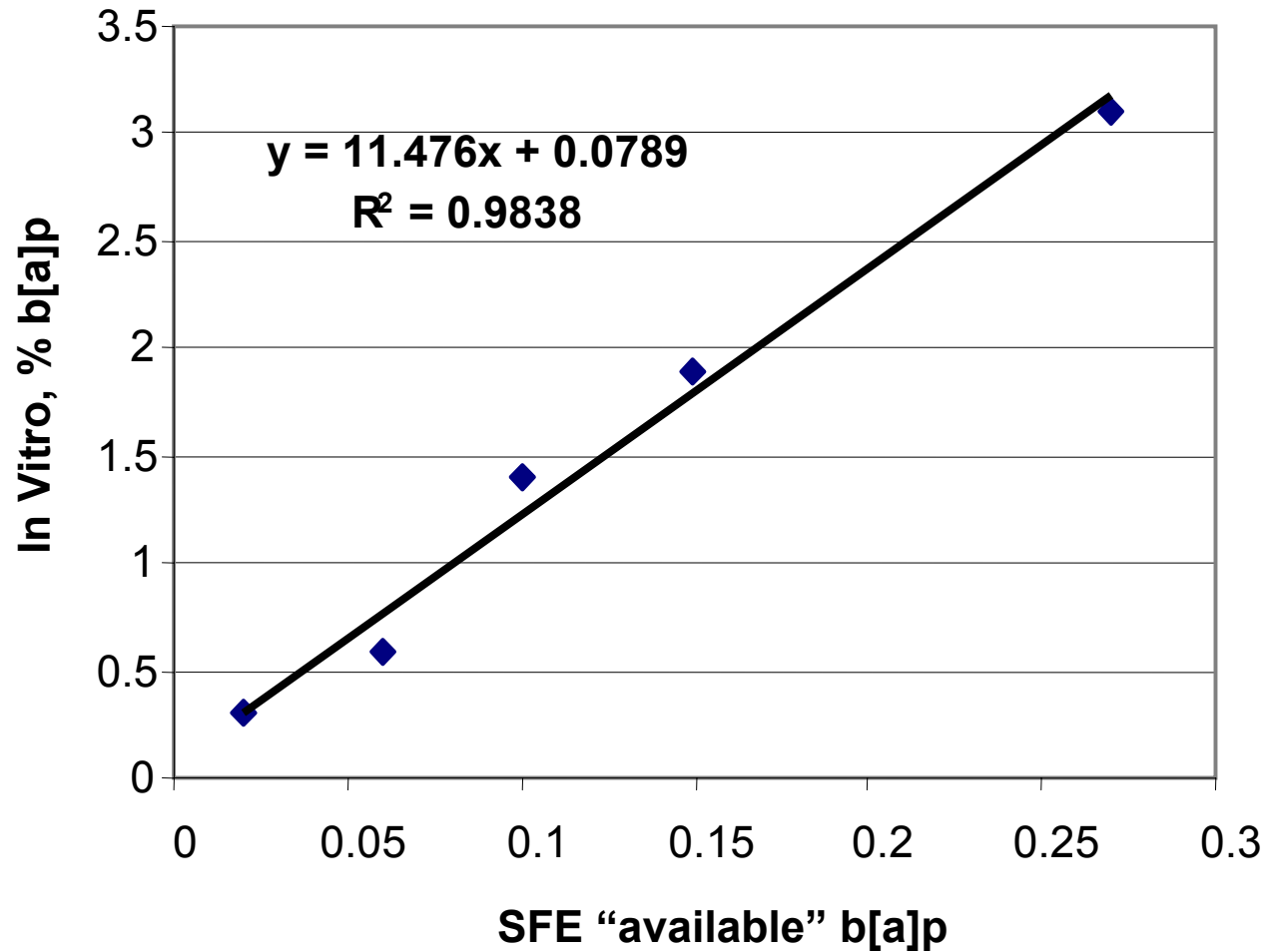
SFE "available" b[a]p

Test case 5: Live worms/dead worms

Removal of SFE-available molecules eliminates toxicity, even though PAH concentrations are still very high.

	Before mild SFE	*After mild SFE*
2-ring	*6080 mg/kg*	*410 mg/kg*
3-ring	*4320*	*850*
4-ring	*5740*	*2910*
5-ring	*2590*	*2410*
6-ring	*1500*	*1500*
Total PAHs	*20200*	*8120*
Earthworms	*100 % dead*	*95 % alive*
Enchtraeid	*100 % dead*	*100 % alive*

Test Case 6: Sediment PAH toxicity

28 day *Hyalella azteca* survival & growth

Compare EPA-predicted toxicity to toxicity based on:

1. **SFE "available" concentrations**
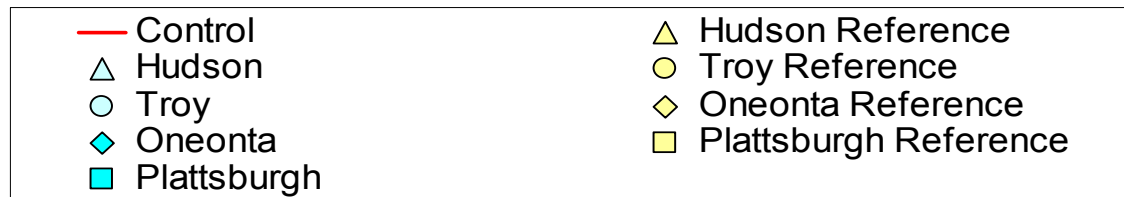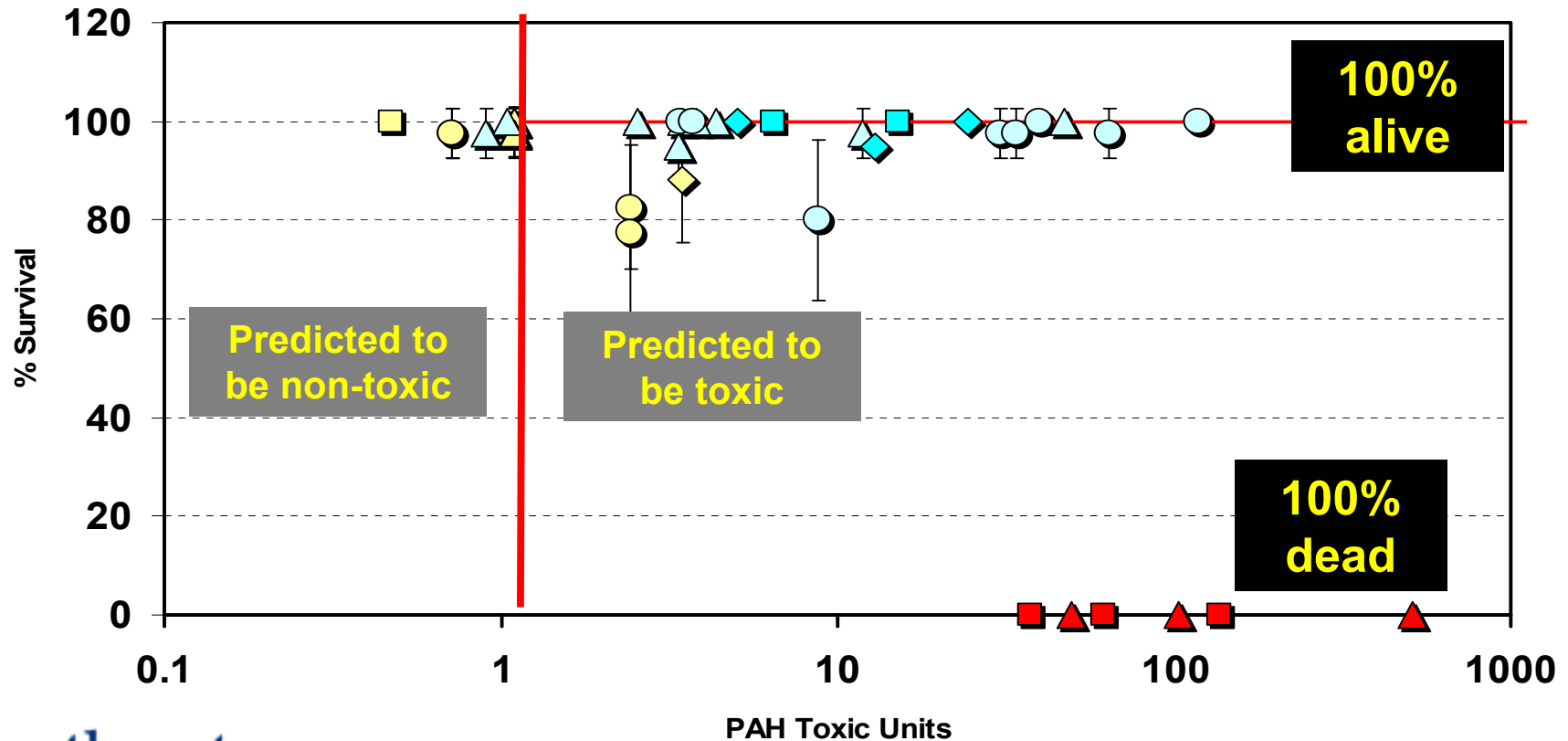2. **Sediment pore water concentrations**

>> *pg/mL detection limits required (part-per-trillion)*

>> *1mL samples desirable for practical sampling limitations*

With proper care for blanks, SPME can yield pg/mL from 1 mL.

*(note, SPME is used for determining water concentrations, not as an availability assay)*

EPA Protocol Using Total PAH Concentrations Overpredicts Toxicity to Hyalella, and Does Not Differentiate Toxic and Non-Toxic Sediments

SFE "Available" Concentrations (rather than EPA total concentrations) differentiates toxic from non-toxic sediments. (43 Sediments, 4 sites)
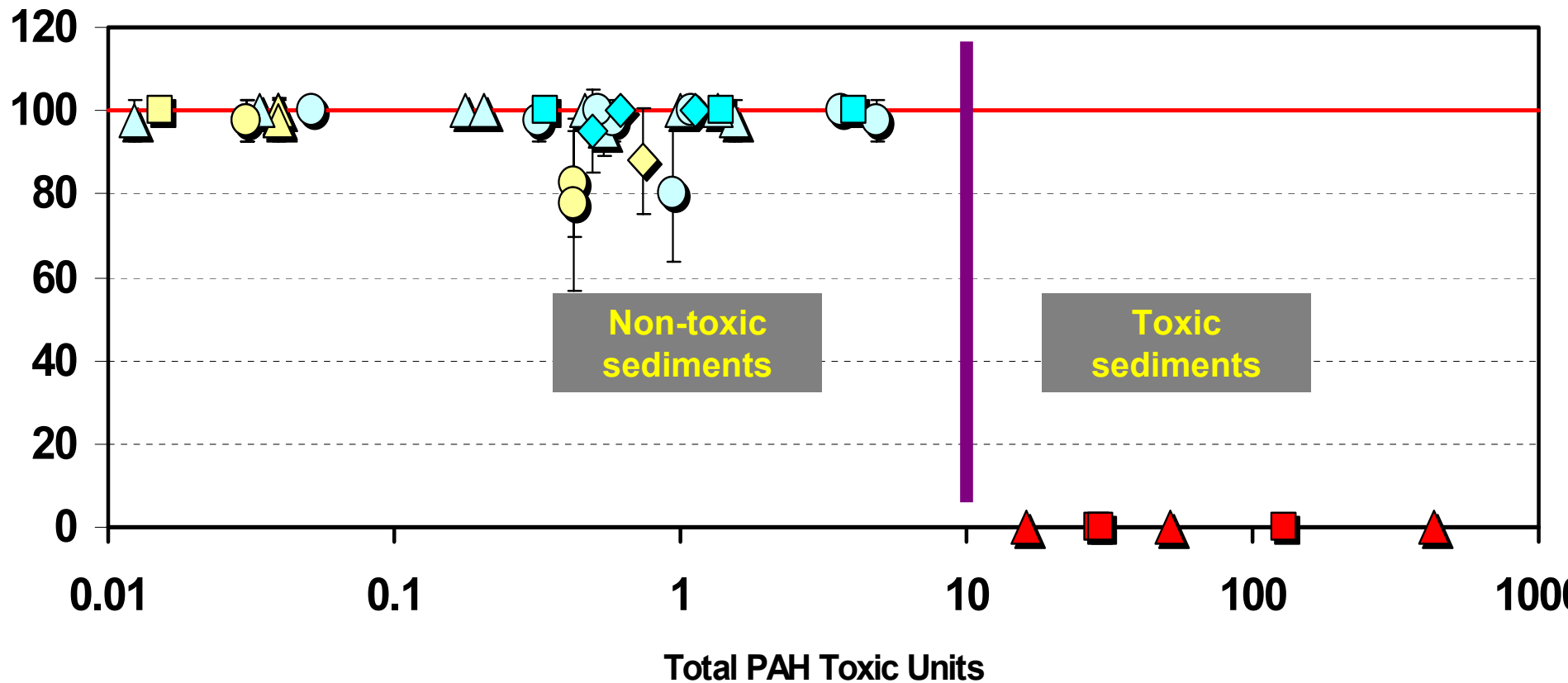
SPME (Pore Water) "Available" PAH Concentrations Differentiates Toxic and Non-Toxic Sediments
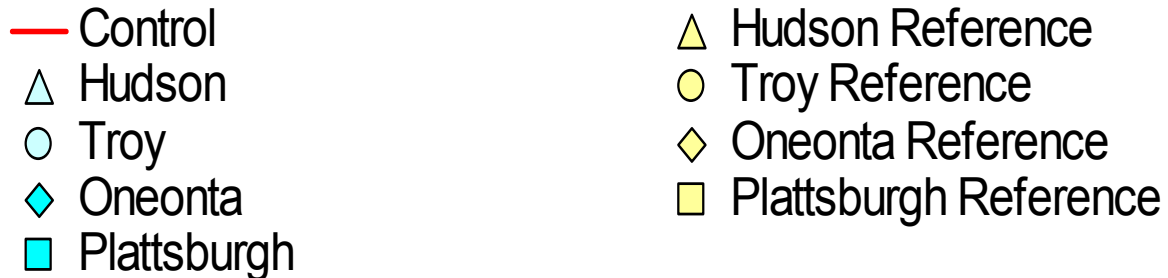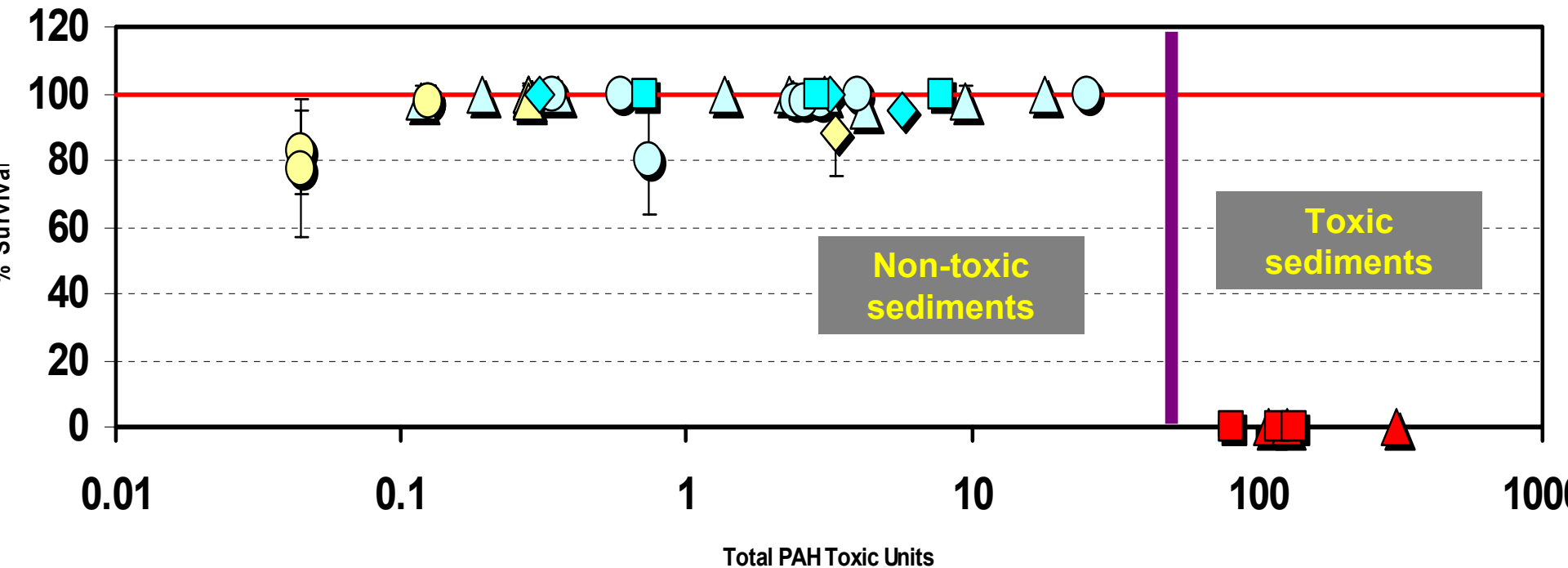
Conclusions

- *Mild SFE-available PAHs improves prediction of biological effects compared to regulatory models.*

- *SFE is appropriately conservative.*

- *Pore water and SFE are predictive for sediments.*

- *Based on:*
  - *2-6 ring PAHs; low mg/kg to wt.% concentrations; soils, sediments, and soots; 2 to 87% TOC*
  - *Prediction of field-bioremediation success (300 days vs. 40 min)*
  - *Agreement with water desorption (120 days vs. 120 min)*
  - *Improved prediction of earthworm uptake*
  - *Correlation with in-vitro mice assay (b[a]p)*
  - *Accurate prediction of Hyalella toxicity (from sediments)*
    - *(Lumbriculus uptake from sediments is in progress)*

Collaborative Studies

Joe Kreitinger (RETEC)   worm (etc.) uptake
            and Martin Alexander, Cornell U.   and toxicity

Ray Loehr and Dustin Poppendieck, U. of Texas, Austin   water vs. SFE "Fs"

Upal Ghosh and Dick Luthy, U. of Maryland (Stanford U.)   single particle Kds

Hoi Ying Holman, Lawrence Berkeley Labs   SFE vs. rats

Roman Lanno, U. of Ohio   SFE vs. dead worm

Joop Hermens, Utrecht U. (Netherlands)   SFE vs. SPME

Frank Doherty   (AquaTox)   sediment biology

Hans Stroo and Dave Nakles, RETEC   industrial relations

Single Particle (mg) K_D Values and Desorption Rates (with Upal Ghosh)
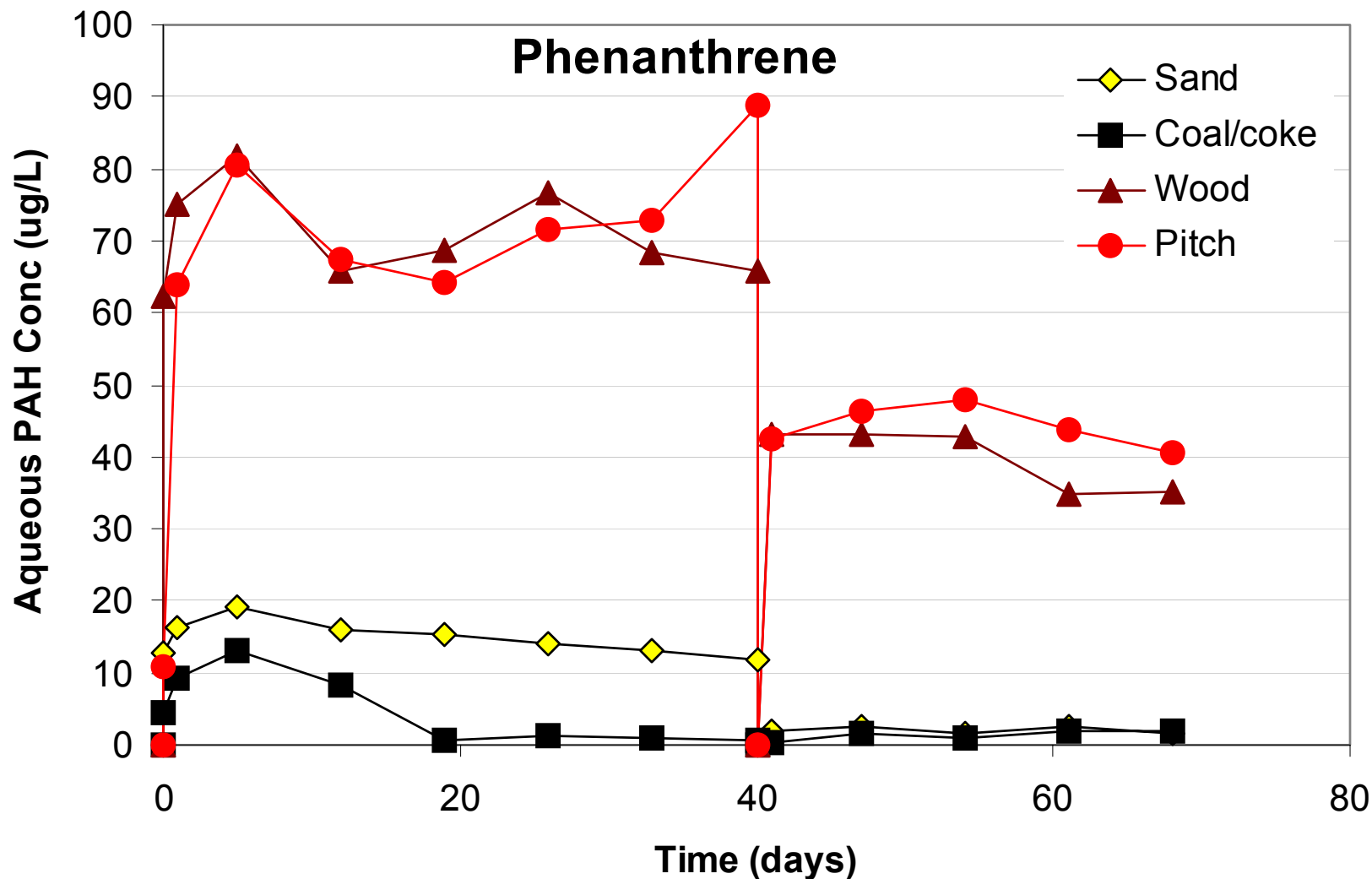
Related Peer-Reviewed Publications

- PAHs in MGP samples

  Environ. Sci. Technol., **2000**, vol. 34, 4103-4110 (Hawthorne et al.)
  Environ. Sci. Technol., **2001**, vol. 35, 4577-4583 (Hawthorne et al.)
  Environ. Sci. Technol., **2002**, vol. 36, 4795-4803 (Hawthorne et al.)

- BTEX in MGP samples

  Environ. Sci. Technol., 2003, vol. 37, 3587-3594 (Hawthorne et al.)

- PCBs in soils and sediments

  Environ. Sci. Technol., **1999**, vol. 33, 2193-2203 (Bjorklund et al.)
  Environ. Sci. Technol., **1999**, vol. 33, 2204-2212 (Pilorz et al.)
  Environ. Sci. Technol., **1999**, vol. 33, 3152-3159 (Hawthorne et al.)